STUDIES ON THE PHYSIOLOGY OF CORALS

V. THE EFFECT OF STARVATION IN LIGHT AND IN DARKNESS ON THE RELATIONSHIP BETWEEN CORALS AND ZOOXANTHELLAE

BY

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WITH SIX TEXT-FIGURES AND THREE PLATES

CONTENTS

1. INTRODUCTION AND LITERATURE.

As already briefly stated in the Discussion to Paper I of this series, there is a great controversy about the food of the reef-building Madreporaria. Some authors believe that the zooxanthellæ form a part, at least, of the food; others maintain that the corals

feed exclusively on other animals, principally zooplankton. The further elucidation—and possible final solution—of this fundamental problem formed one of the principal objects of the expedition.

Boschma (1924, 1925*c*, 1926) has given an adequate review of the evidence on both sides. The major protagonists on the one side have been Murray (1889), Krämer (1897), Duerden (1902, 1906), Carpenter (1910), Vaughan (1912, 1919) and Mayer (1918), all of whom were of the opinion that corals live exclusively on zooplankton. The earlier authors (Murray and Krämer) based their views largely on the presence, in their opinion, of adequate supplies of zooplankton in the waters around coral reefs, and the later authors on experimental evidence that corals feed on animal matter (exclusively, as Vaughan [1912] showed). The opposite view was held by Gardiner (1899, 1901, 1902–3, 1904, 1928), Gravier (1908, 1913), Walther (1919), and received qualified support from Hickson (1906, 1924). All of these authors considered that there was either too little zooplankton in the water to satisfy the needs of the corals, or else that the almost invariable absence of such food within the coelentera of corals demonstrated that such food could not be—certainly was not—caught in the requisite quantities.

The fallacies in the last of these arguments have already been fully demonstrated in Papers I and II of this series, where it was shown that corals are highly specialized carnivores, with feeding mechanisms especially adapted for the capture of living zooplankton, and with digestive enzymes equally specialized for the breaking down *exclusively* of animal matter.

The controversy has, of recent years, been intensified as a result of the publication of a series of papers by Boschma, giving the results of his observations and experimental work on a variety of Madreporaria in the East Indies (1924), on various Atlantic corals and other Coelenterata at Bermuda (1925*a*, 1925*b*), on *Astrangia danae* at Wood's Hole (1925*c*), and on the actinian *Cribrina xanthogrammica* at La Jolla in California (1926). This author has produced experimental evidence in support of his view that corals and allied coelenterates can actually *digest* zooxanthellae.

Boschma based his conclusions largely on the presence in exceptionally large numbers of degenerating zooxanthellae in the "absorptive' zone of the mesenterial filaments. He also found that they were most numerous in this region when the animals were starved, and least abundant when they were repeatedly fed with meat. His conclusions can best be stated in his own summarizing paragraph (1926, pp. 996–7):

"The food of reef-corals and of actinians which live in association with zooxanthellae consists for a part of these zooxanthellae and for another part of animal matter. The polyps try to get as much animal matter as possible, but in case of starvation they depend chiefly upon the zooxanthellae. The surplus of the rapidly multiplying zooxanthellae in the tissues is removed from the entoderm cells to the gastric cavity, and, as far as needs may be, these algae are digested or removed through the mouth."

It has already been shown in Papers III and IV that the presence of zooxanthellae, some of them in a state of degeneration, within the "absorptive" zone of the mesenterial filaments, does *not* imply that the algae are being digested there. It is every bit as probable that they are being *excreted* from the tissues. According to Boschma they are first expelled (he does not say exactly where) into the coelenteron and then, if other food is lacking, they are re-ingested and digested within the "absorptive" zone of the mesenterial filaments. He makes no comment on this remarkable change of habit in an animal

normally exclusively carnivorous. It has been abundantly demonstrated in the preceding paper that, if the metabolism of the corals is lowered by any agency, zooxanthellae are expelled in great numbers, but no evidence has been produced indicating that they are ever re-ingested. As for their digestion, there is the negative evidence recorded in Paper II (see p. 74 of this volume) that zooxanthellae are not attacked by extracts of the mesenterial filaments even after 40 days' incubation. Vaughan has recently (1930) published the results of an experiment carried out by him in 1912 on *Macandra areolata*. A specimen was placed in filtered sea-water for five days, and at the end of this period of starvation " its tissues had become thin, emaciated and pale." The animal was then placed under normal conditions and repeatedly fed with animal food. " Ten days of such feeding were sufficient to restore the colony to the condition that it was in at the beginning of the starvation experiment." Vaughan very naturally asks why the coral should so quickly show the effect of starvation if the zooxanthellae are of so much value as food, and why so much animal food was required " to restore it to its condition prior to its starvation."

It was clearly of the first importance in this series of researches to repeat Boschma's experiments (that of Vaughan was unknown to us at the time) on as large a scale as possible. Not only was it necessary to extend and confirm evidence already recorded which pointed to the inability of corals to digest zooxanthellae, but it was also necessary to be certain that nutrient material was not passed from the zooxanthellae to the tissues of the coral. Keeble and Gamble (1907) have shown that fat is passed from the symbiotic *Chlamydomonas* to the tissues of *Convoluta roscoffensis*, while Arndt (1913) has produced evidence of a much less convincing character that there is a similar passage of fat from the zooxanthellae to the tissues of the actinian *Heliactis bellis* in which they live.

With these ends in view an elaborate experiment was set up in which a variety of Madreporaria were starved and fed under parallel conditions in both light and darkness. The results of this experiment form the subject-matter of this paper, and the bearing of the results obtained on previous work, and in particular that of Boschma, will be discussed in detail in Section 5.

We received considerable assistance in the practical work involved in this research. Mrs. Yonge carried out the oxygen and phosphate analyses and took certain of the photographs. Mr. G. W. Otter rendered most important assistance both by his very skilful construction of the light-tight box employed in the experiment, by taking photographs and by assisting, on various occasions, in the maintenance of the experiment which involved much time and labour. Miss S. M. Marshall was left in charge of the experiment during our absence for five weeks in the Torres Strait, and frequently assisted by the provision of tow-nettings, which were used as food for the corals. Our sincere thanks are due to these three members of the expedition, without whose help this work could not have been carried through. Finally, the senior author wishes to record his personal indebtedness to Dr. H. Boschma, with whom a continuous correspondence, between Australia and Holland and then the East Indies, was maintained. It has been found impossible to confirm Dr. Boschma's results, but these researches owe much to the friendly and helpful criticisms to which he subjected them during the period in which they were being carried out.

After our return to Great Britain, sections of material fixed in Bouin and Flemming's fluids were prepared by the senior author. These were in all cases cut 6μ thick.

2. DESCRIPTION OF EXPERIMENT.

It was decided at the outset of the expedition to set up, for reasons already stated, an experiment in which corals could be starved and fed under identical conditions in both light and darkness. It was hoped in this way to determine the effect of—

(A) Starvation of the coral alone (*i. e.* starvation in light).

(B) Starvation of the zooxanthellae alone by the deprivation of light (*i.e.* feeding of the corals in darkness).

(C) Starvation of both corals and zooxanthellae (*i. e.* starvation of the corals in darkness).

The conditions observed could then be compared with those in –

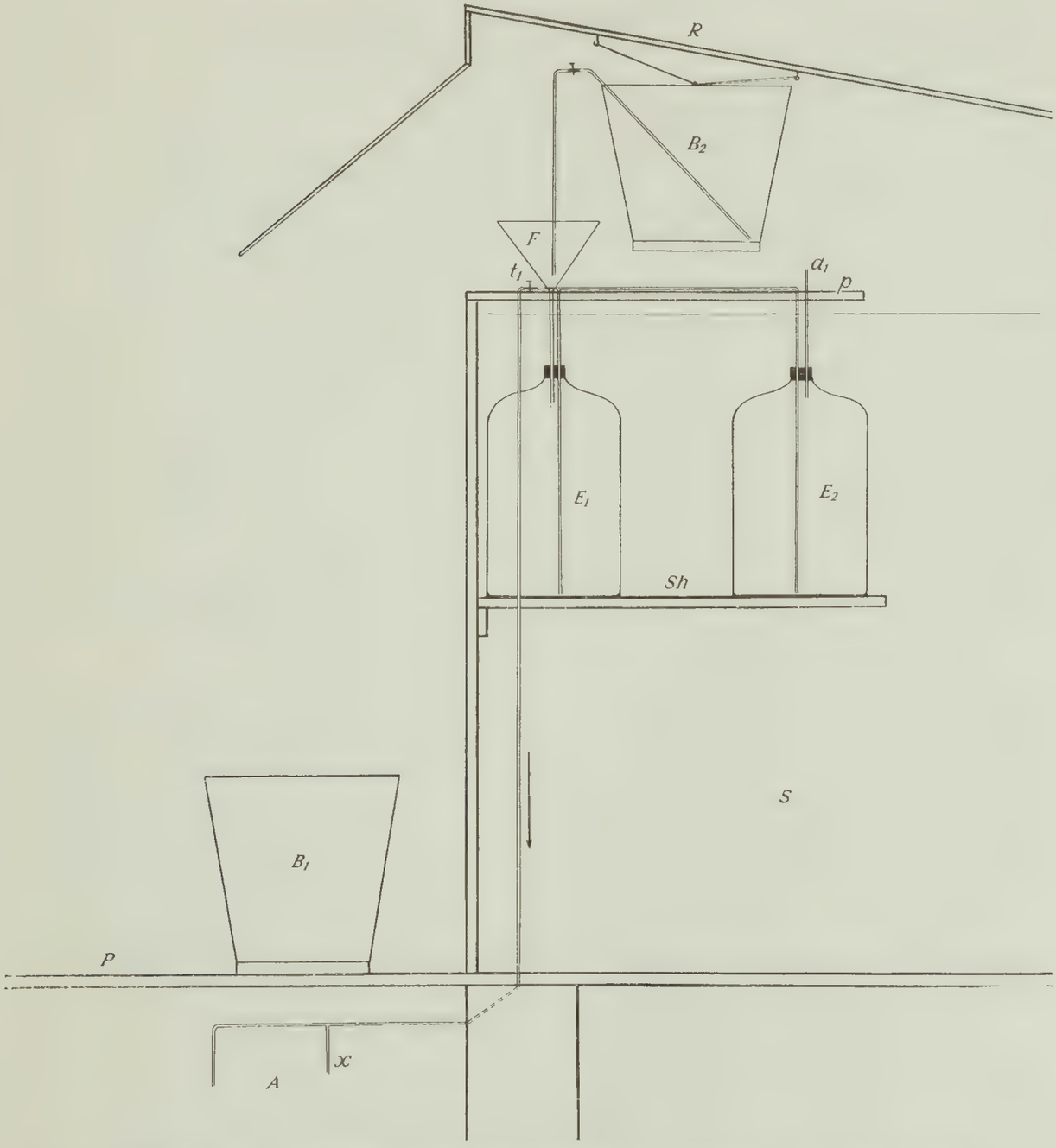
(D) Corals kept under, as far as possible, normal conditions (*i. e.* feedings of the corals in light).

Originally it was hoped that it would be possible to accomplish this in glass jars put out in the sea in a specially constructed wooden crate, the contents of the jars being examined at frequent intervals. Experience soon showed that this was quite impracticable, and that the only satisfactory method was to set up an experiment in the aquarium. This was situated behind the laboratory hut and, as briefly recorded in the introductory paper to these reports (p. 6 of this volume), was formed by the enclosure of the space beneath the sea-water tank (see Plate I, fig. 2). The area so enclosed was about 8 ft. high, and was fitted with a stout bench, 3 ft. from the ground, and with shelves above that. The main body of this aquarium, directly beneath the sea-water tank, was used for general purposes, but the smaller section, under the platform which projected outward on the east side of the sea-water tank (and which is shown in Plate I, fig. 2) was given over entirely to this experiment.

Various initial forms of the experiment failed, and it was not until November, 1928, that the various problems encountered in connection with the maintenance of corals under these experimental conditions were all satisfactorily solved. After this period the experiment was maintained in continuous operation until the termination of the expedition, minor improvements suggested by experience alone being made from time to time. The general appearance of the experiment as finally elaborated is shown in Plate I, fig. 1. The detailed description of it will best be followed by continual reference to Text-figs. 1 and 2.

The necessary supplies of sea-water were obtained regularly twice daily, at about 9 a.m. and 6 p.m. Two large galvanized iron buckets ($B._1$) and one large earthenware jar ($E._3$) were filled in the open waters of the anchorage from the dinghy. The buckets of water were carried to the platform (*P.*) besides the sea-water tank (*S.*). Here a portion of their contents was transferred to a smaller bucket ($B._2$), which was then slung from the sloping roof (*R.*) of the tank, as shown in Text-fig. 1. A siphon of glass tubing was so arranged that the water was drawn off into a large filter funnel (*F.*) in which a clean, coarse filter-paper had previously been placed. The water filtered through this into a large earthenware jar ($E._1$), which, when full, siphoned over into a second jar ($E._2$). Both of these jars stood on a shelf (*Sh.*) constructed, as shown, within the sea-water tank (*S.*), so that the jars were covered with water when the tank was filled every evening. Any risk of this water entering the jars was effectively overcome by inserting tightly-fitting corks and covering them with a layer of marine glue. At all times the jars and their contents

were kept cool, which was the object aimed at. Above the jars a small platform (*p.*) was fitted on the top of the wooden sides of the tank, through openings in which passed the stem of the filter funnel and other glass tubes. The second jar (E_2), beside being



TEXT-FIG. 1.—Diagram of the system for the initial filtration and the storage of the water used for circulation over the starved corals in the experiment. × ½. For explanation of lettering see text.

connected with the first, had an air outlet (a_1), to the end of which a tube could be attached by means of rubber tubing and, by suction on this, the siphon between the two jars could be established before the first jar was full. This was usually done. The bucket (B_2)

was continuously re-filled from the larger buckets by means of a metal dipper until the original supply of water had been exhausted.

The screw-clip ($t._1$), previously kept closed so as to maintain the integrity of the siphon between the two jars, was now opened, and in this way connection was made between the contents of the jars and the experiment in the compartment (*A.*) beneath the platform. As indicated by the arrows, the water flowed downward through the length of glass tubing which ran down the outer side of the sea-water tank, and then turned inward along the corner of the roof of the aquarium (this section is indicated by the broken lines in Text-figs. 1 and 2). It then continued along the top of the back wall of the aquarium, as shown in the text-figures. The siphon was established by inserting a glass tube into the rubber tubing on the end of the pipe marked *x*, opening screw-clip $t._2$, closing screw-clip $t._3$, and then sucking the water over from the reservoirs above. As soon as the water appeared and filled the tubes, $t._2$ was immediately tightly screwed up again. A glass jar (*Jx.*) stood on the shelf beneath this pipe, and in it collected any slight escape of water.

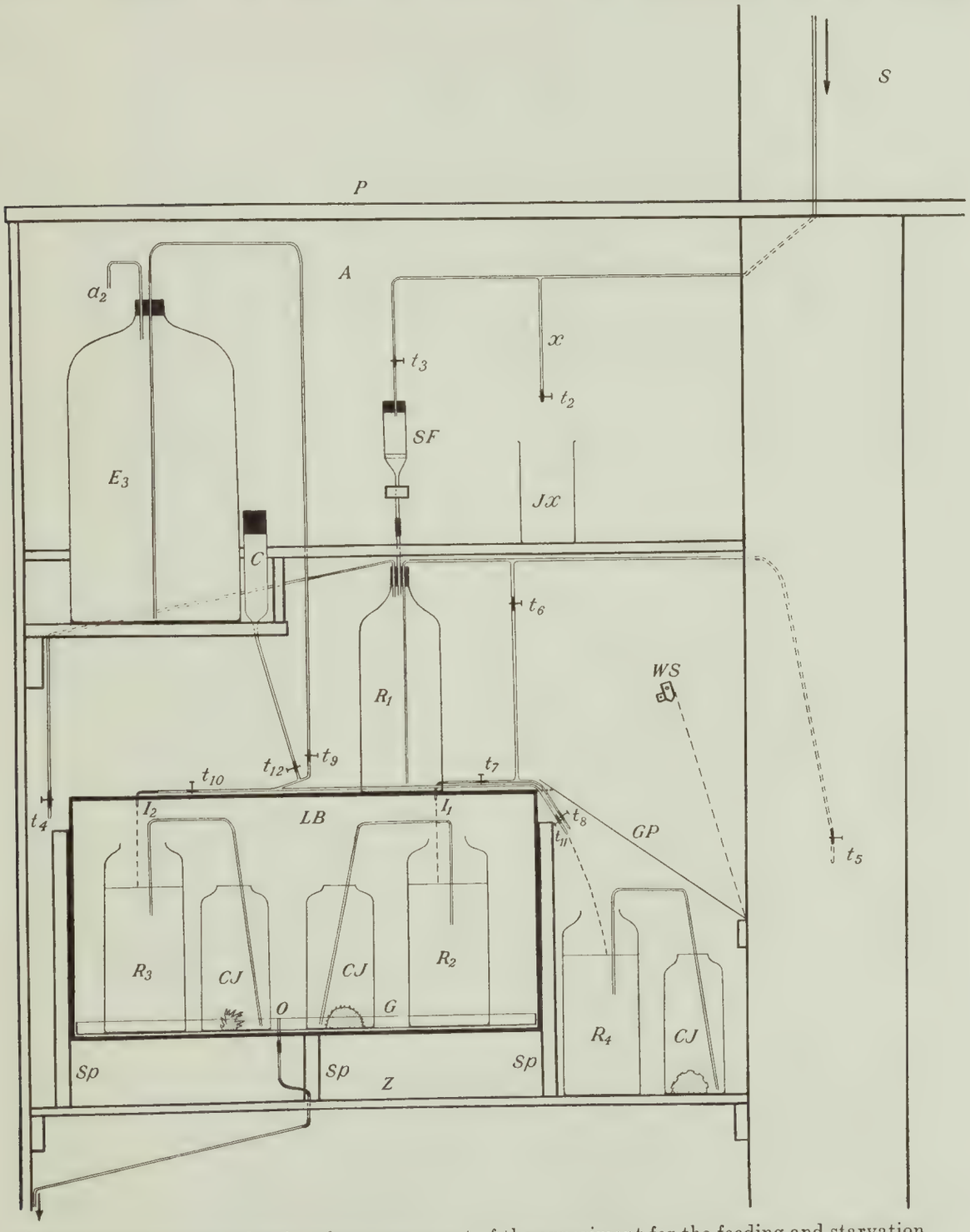
The supply of water from the reservoirs in the tank above, and which had already been filtered once through a coarse filter-paper, was filtered a second time through a sintered silica filter (*S.F.*) of medium texture. When the siphon was established the screw-clip $t._3$ was opened, and the water emerged into the filter funnel with considerable force owing to the height of the reservoirs above it. The top of the funnel was closed by a large, tightly-fitting rubber cork, through which the glass inlet pipe passed. Filtration usually took place at considerable speed at first, but gradually slowed down as material was deposited on the upper surface of the filter. It was usually necessary to substitute a clean filter each day, the previous one being removed and thoroughly cleansed with dichromate sulphuric, which was drawn through it under pressure.

After this second filtration, which effectively removed any fine zooplankton which had passed through the first filter, and also probably all the phytoplankton, the water passed into a reservoir jar ($R._1$), consisting of a half winchester bottle. While this was filling, the vulcanite tap, $t._4$, at the end of the long air outlet was kept open. As soon as the jar filled, the water flowed along this tube and out of the opening, when the tap was at once closed. When the reservoir jar was full and there was a head of water from above, it was always possible to draw off the twice filtered water used for a variety of experiments, many of them described in Paper IV, through this tap. It there was no head of water, the contents of the jar itself could be siphoned off by way of the tube terminating on the right-hand side of the compartment in the tap, $t._5$, which was fastened to the inner of the two pillars which supported the one end of the sea-water tank.

The great bulk of this water was, however, used in the experiment. As soon as the reservoir jar was full, screw-clip $t._6$ was opened and the water flowed down to the level of the top of the light-tight box (*L.B.*), where it bifurcated, one branch, controlled by screw-clip $t._7$, passing through the top of the box at $I._1$, and the other, controlled by screw-clip $t._8$, over the edge of the box. When these two clips were opened water flowed into the secondary reservoir jars, $R._2$, inside the box and one *behind* $R._4$, respectively. It is now necessary to make a short digression in order to describe the nature of the light-tight box.

This essential constituent of the experiment, constructed, as already stated, by Mr. G. W. Otter, consisted of a stout wooden box, the appearance of which *in situ* is well shown in Plate I, fig. 1, and its various parts in Plate I, fig. 3. It was 1 ft. high, 1 ft. 11 in. long and 1 ft. 3 in. deep, and stood on wooden supports (*Sp.*), one at each side and one at the

middle in the back, which raised it $3\frac{1}{2}$ in. above the bench (Z.) on which it rested. It was secured by screws to the back of the aquarium. Both inside and out were painted



TEXT-FIG. 2.—Diagram showing the arrangement of the experiment for the feeding and starvation of corals in light and in darkness. $\times \frac{1}{8}$. For explanation of lettering see text.

black, and all junctions between boards were covered with strips of wood, the whole being absolutely light-tight when the front was in place. The front, shown in Plate I, figs. 1

and 3 (left-hand side), but not in Text-fig. 2, was detachable. It pushed on over the rest of the box and fitted very tightly, having to be prized off by some metal instrument. There was a small circular opening near the centre -designed for observational purposes, but seldom used—closed by a well-fitting cork. Both the front and the top of the box were covered with galvanized iron sheeting. Within there rested on the bottom a galvanized iron tray (*G.*) (shown on the right in Plate I, fig. 3), with sides about $\frac{1}{2}$ in. high and with a central metal outlet pipe (*O.*) of the same height, the lower end of which projected through the under side of the box. The two inlet pipes, $I._1$ and $I._2$, passed through the roof of the box and were secured, and the openings made light-tight, by a surrounding of marine glue. The glass tubes themselves were painted black for some distance back from the inlets, so as to prevent any entrance of light by this agency. The box could accommodate the two secondary reservoir jars, $R._2$ and $R._3$, both 4 in. in diameter, and also twelve smaller jars for corals (*C.J.*), all 3 in. in diameter, and gave complete satisfaction throughout the course of the experiment.

Reverting to the description of the experimental procedure, the water which flowed through the reservoir jar ($R._1$) supplied the two sets of corals, which were starved in the dark and in the light. These were situated, respectively, in the right-hand half of the box, and at the back of the bench to the right of the box. The water poured into the secondary reservoir jars and was siphoned off from them into the smaller jars (*C.J.*). This procedure ensured that all jars received an equal share of the water, and were at the same time separated from one another so that the death of any coral did not affect the water surrounding any of the others. This procedure, elaborated as the result of various preliminary experiments, proved highly satisfactory, and is to be recommended for other experiments of this type. The glass siphon tubes took water from the middle of the reservoir jars (thus allowing sediment to settle to the bottom in the case of the unfiltered water, which will be discussed shortly) and discharged it at the bottom of the jars containing the corals. This ensured a thorough removal and aëration of the water surrounding each of the corals twice daily. The water which overflowed from these smaller jars made its way on to the bench, and so, through cracks, into the sand beneath, in the case of the jars in the light. In the box the overflow collected in the metal tray (*G.*), and when this filled was drawn off through the outlet pipe (*O.*), and thence through the bench beneath and to the outer side of the aquarium, where it also discharged into the sand.

The starved corals, even in the darkness, received ample aëration and renewal of water, as was shown by their survival, in certain cases for many months, under these conditions. A full supply of water, two large bucketsful, were sufficient to maintain a steady flow through the experiment for about one hour.

The contents of the earthenware jar ($E._3$), which was filled at the same time as the two buckets, were not filtered, but were used for circulation over the fed corals in both light and darkness. The jar was placed on an especially constructed shelf, the cork with a short air inlet ($a._2$) and a siphon tube reaching to the bottom of the jar being first inserted. Connection was then made between the siphon tube and the discharge tube, which was secured to the back of the aquarium, by means of rubber tubing, and the siphon was started by attaching a glass tube to the end of the air inlet ($a._2$) and blowing, the screw-clip $t._9$ having first been opened. When the screw-clips $t._{10}$ and $t._{11}$ were both open the water flowed through into the secondary reservoir jars $R._3$ and $R._4$, in the dark and the light respectively. The stream of water was in this case more powerful, owing to absence of

the restraining influence of the silica filter, and the supply of water was smaller. The earthenware jar was emptied in approximately a quarter of an hour. Experience showed that a short rapid flow was better than a longer and correspondingly slower flow. This supply of water twice daily proved ample for the demands of the fed corals.

These corals were fed every second day in the evening. Tow-nettings were collected in the anchorage after dark (plankton was naturally scarce in the surface waters during the day), and the catch filtered through very coarse bolting-silk. In this way the larger organisms, which might have blocked the pipes or quickly fouled the water, were removed, while great numbers of smaller organisms, largely copepods, and so ideal food for corals, remained in the water. This was divided into two equal portions. One of these was divided amongst the jars containing the corals fed in the light. The other was placed in the container (*C.*), which was secured near the base of the earthenware reservoir ($E._3$). The cork which normally blocked the entrance to this was first of all removed, and the screw-clip $t._{12}$ was opened; $t._{10}$ was also opened, but $t._9$ and $t._{11}$ were both shut. The water and plankton in the container thus ran down into the reservoir jar $R._3$ in the light-tight box. As soon as the container was empty the screw-clip $t._9$ was opened, and water was allowed to flow into the box and also to rise into, and so rinse out, the container; $t._9$ was then again shut and the container was finally drained, after which $t._{12}$ was screwed up, and $t._9$ opened to allow a continuous flow of water for a few minutes through the section of the box containing the fed corals. In this way the plankton was distributed through the various jars containing corals inside the box. As will be demonstrated clearly later in this paper, these fed corals, both in the light and in the dark, showed clearly that they received adequate supplies of food. As shown in Text-fig. 2, these corals were kept in the left-hand side of the light-tight box, and in the front of the bench to the right of the box.

It was necessary to clean out the jars containing the fed corals, and also the reservoir jars $R._3$ and $R._1$, not less than twice weekly owing to the collection of dead plankton and sediment. The jars containing the starved corals were frequently examined, but only cleaned out at infrequent intervals—approximately once a fortnight. Contamination of the water in the jars containing the corals in the light by water dropping from the shelf above was prevented by the interposition of a slanting glass plate (*G.P.*), the higher side of which rested against supports, which projected from the upper edge of the light-tight box. During the examination of the corals this glass plate was turned back and secured by a wooden stop (*W.S.*), its new position being indicated by the broken line in Text-fig. 2.

The experimental procedure as outlined above proved highly satisfactory. The aquarium was perpetually in the shade, and so the corals were maintained at a uniform temperature, which varied from about 18° C. in the winter to 25° C. in the middle of the summer. The water from the various reservoirs was always of a similar temperature when it reached them. The shade in which the corals kept in the light invariably lived was, perhaps, a little too intense exactly to reproduce conditions on the surface of the reefs, but, as will be demonstrated in the succeeding sections of this paper, corals fed in the light certainly acted as effective controls to the others. They were thus clearly living under conditions not very dissimilar to those to which they were normally exposed.

3. RESULTS OF EXPERIMENTS ON VARIOUS MADREPORARIA.

Small coral colonies or portions of colonies, all of them previously cleaned as far as possible, were used in the experiment. A variety of different genera were experimented upon. Some proved highly satisfactory, in others there was a considerable mortality, but eventually specimens were secured which gave satisfactory results, while with others, again, no success was ever attained. The latter category included all species tried of *Pocillopora* (adult colonies), *Acropora*, *Montipora* and *Porites*. All of these died very quickly, even in the light. The explanation in the case of *Acropora* and *Porites* appeared to be connected with the thick layer of mucus which invariably formed over their surfaces, and of which, in the absence of powerful water movements, they were unable to rid themselves. This is in agreement with the findings of Marshall and Orr on the effect of sediment on corals, reported in this volume, and also agrees with the results of the experiments on corals kept in the light-tight box on the reef flat which were described in Paper IV of this series. There *Acropora* always quickly died, and species of *Porites*, though they survived for 152 days, showed by their low excretion of phosphorus that they were in poor condition. The species of both genera employed were all taken from the surface of the reef. *Pocillopora* and *Montipora* did not form this coating of mucus, but here again their failure to survive agrees with our previous findings and those of Marshall and Orr. All these four genera of corals possess small polyps and, as emphasized in the discussion to Paper I, their ciliary mechanisms are less efficient as cleansing agents than those of the deeper water corals with larger polyps, which live under conditions where no assistance in cleansing can be obtained from water movements. Indeed, in the case of *Pocillopora* and *Porites* cilia definitely assist in feeding.

It was surprising to find that a similar type of coral, *Psammocora gonagra*, was the most successful of all, and that *Cyphastrea chalcidicum* also lived well under experimental conditions. The latter has somewhat larger polyps, while *Psammocora* is undoubtedly an exceptionally hardy coral, but it is clear that much work remains to be done before the conditions controlling the distribution of the different genera and species of reef-building corals are fully understood.

Experiments were carried out on *Millepora* which lived well under experimental conditions. After two months material from each of the four colonies was fixed in formol, decalcified and examined. It was seen that the zooxanthellae were much more numerous in the colony which had been fed in the light than in the others; those from the colony starved in the light showed evidence of degeneration *in situ*. Material fixed in Bouin and Flemming, and subsequently sectioned, failed to give satisfactory results, and, in view of the probability (unsuspected, unfortunately, at the time the experiments were carried out) that the zooxanthellae in *Millepora* are different from those in the Madreporaria and the other Anthozoa (see Paper IV), it is impossible to come to any definite conclusion as to the results of experiments carried out with *Millepora*. There does, however, appear to be a possibility that conditions here are different from those which prevail in the Madreporaria.

The results of experiments with the madreporarian genera, *Fungia*, *Goniastrea*, *Psammocora*, *Galaxea*, *Cyphastrea*, *Lobophyllia*, *Pocillopora* (newly-settled colonies) and *Dendrophyllia* all gave interesting and satisfactory results, and these will be discussed in the above order,the results being tabulated as far as possible.

(i) *Fungia danai.*

A. *Starved in Light.*

No.	Date.	Remarks.	Died.	Period in experiment.
A1	15.x.28	*After* 28 *days* found extruding brown masses through mouth, thence off disc. These consisted of mucus and zooxanthellae, the great majority of which were dead, reduced in size or irregular in shape	..	73 days.
		After 73 *days* disc tissue reduced to strip 1 cm. wide round rim (see Text-fig. 3 and Plate II, fig. 4), skeleton and mesenterial filaments exposed within		
		Transferred to light-fed conditions, but died after 16 days, showing some signs of regaining tissue of disc		
A2	5.xii.28	*After* 9 *days* disc tissue found retreating appreciably from mouth, and zooxanthellae being extruded in large numbers	6.i.29	32 days.
A3	,,	Ditto	,,	32 ,,
A4	9.i.29	*After* 28 *days* disc tissue greatly reduced and ruptured in many places, also beneath the skeleton. About 0·5 cm. of skeleton exposed around mouth (see Plate II, fig. 5). Great numbers of zooxanthellae extruded	5.iii.29	55 ,,
A5	24.i.29	*After* 7 *days* zooxanthellae being extruded		
		After 52 *days* (at death) tissues very emaciated and broken down in many places, but no retreat of disc from mouth in this case; instead a general breaking down with mesenterial filaments exposed everywhere, on surface, sides and beneath skeleton	17.iii.29	52 ,,
A6	26.iii.29	*After* 16 *days* zooxanthellae being extruded, tissues greatly emaciated, but not retreating appreciably from mouth, everywhere perforated	18.iv.29	23 ,,
A7	,,	Ditto	,,	23 ,,

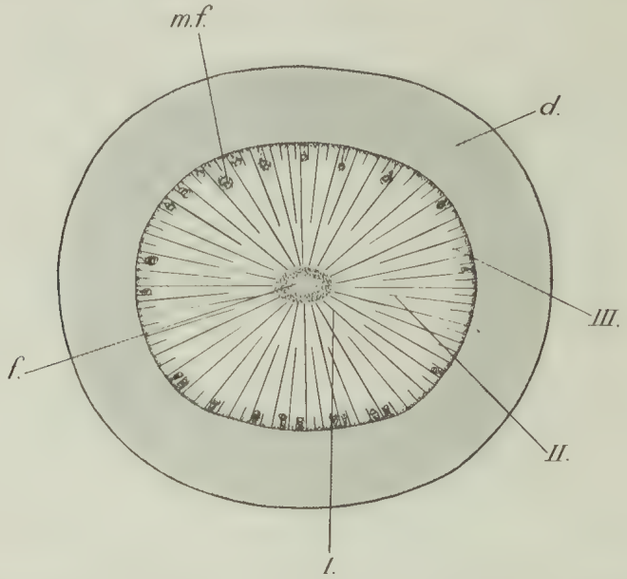
In addition to the above, 9 other *Fungia*, making 16 in all, were experimented upon. Of these, one lived for 4 days, two for 5, one for 14, two for 16, one for 24, one for 31 and one for 32 days.

An examination of the results tabulated above reveals at once that the corals showed the effects of starvation very quickly, and that *their first response was to extrude zooxanthellae in very great numbers.* The majority, though not all, of these were dead when examined; approximately 25% appeared to be still alive. The second response to starvation was *reduction of the tissues.* This is particularly obvious in a coral such as *Fungia*, and was clearly seen even after 9 days' starvation in the case of A2 and A3, and, as shown in Plate II, fig. 5, was well marked in A4 after 28 days of starvation. At the same time the tissues became much paler owing to the loss of many of the zooxanthellae.

The results obtained with A1 were especially striking. The actual appearance of this coral after being starved for 73 days is shown in Plate II, fig. 4, but Text-fig. 3, which shows the actual dimensions of the coral and the extent of its disc tissues (*d.*), demonstrates more clearly the great reduction of the latter. The length of the longer axis of the disc was 6·5 cm., and of the shorter 5·8 cm. This was originally covered with tissue which had shrunk to a rim 1 cm. wide (*d.*), the central fossa (*f.*), and the first (I), second (II) and beginning of the third (III) cycle of septa with mesenterial filaments (*m.f.*) between them, being widely exposed.

It will be seen that *Fungia* fed in the darkness (B.) live for long periods under these experimental conditions. Neither B1 nor B2, which were in the light-tight box for 111 and 165 days respectively, died natural deaths. The effect of darkness on the zooxanthellae is plainly shown. These are killed by starvation due to the inability of

their chlorophyll to form carbohydrate in the absence of light. As a result of this the tissues of the corals become even paler than those of *Fungia* starved in the light, but they remain throughout *intact and healthy*, as shown in Plate II, figs. 6 and 7.



Text-fig. 3. *Fungia danai*, diagram showing the reduction of the tissues of the disc in specimen A1 after it had been starved in the light for 73 days. Nat. size. *d.*, disc tissue ; *f.*, central fossa ; *m.f.*, mesenterial filaments ; *I* , *II.*, *III.*, first, second and third cycle of septa.

The difficulty of keeping *Fungia* when starved in darkness (C.)—deprived of light as well as of food—was a little greater than keeping them starved in the light. A good criterion of the ease or otherwise of keeping *Fungia* under the various experimental conditions is provided by the total of animals used in each series of experiments, which amounted to 16 in A, 4 in B, 20 in C and 3 in D. Otherwise the results of series C closely resembled those recorded for series A, except that the zooxanthellae, as in series B, were all dead when extruded. Pressure of other work prevented any photograph being taken of the most successful coral, C3, after it had been starved in darkness for 115 days, but its condition closely resembled that of A4, which is shown in Plate II, fig. 5.

B. *Fed in Darkness.*

No.	Date.	Remarks.	Died.	Period in experiment.
B1	5.xii.28	*After* 71 *days* tissues still healthy and *intact*, but much paler, upper side pale buff instead of medium brown colour, under-side almost pure white (see Plate II, fig. 6). Cœlenteron filled with a dark mass of zooxanthellae with a little binding mucus, all dead and degenerating *After* 111 *days* still in excellent condition, but almost colourless, fixed.	..	111 days.
B2	6.xii.28	*After* 70 *days* condition exactly as reported for B1 (see Plate II, fig. 7). Died as result of contamination, not of experimental conditions	20.v.29	165 ,,
B3	26.iii.29	Similar results	20.v.29	55 ,,
B4	,,	,, ,,	4.v.29	39 ,,

C. *Starved in Darkness.*

No.	Date.	Remarks.	Died.	Period in experiment.
C1	9.i.29	*After* 11 *days* tissue retreating from mouth *After* 18 *days* tissue still further from mouth, great numbers of dead zooxanthellae being extruded *After* 27 *days* edge of septa exposed for about 1 mm. all round, zooxanthellae continually extruded and tissues pale	5.ii.29	27 days.
C2	21.ii.29	*After* 9 *days* condition excellent, tissue retreating from mouth *After* 24 *days* septa exposed for 5 mm. around central fossa, zooxanthellae extruded in large numbers	30.iii.29	37 ,,
C3	30.iii.29	*After* 62 *days* tissue very pale and retreating from mouth, masses of dead zooxanthellae being extruded *After* 75 *days* tissues retreated beyond edge of septa, zooxanthellae still being expelled in great numbers ; tissues very pale *After* 86 *days* septa exposed for 4 mm., tissues otherwise intact, but very pale, zooxanthellae still being extruded *After* 115 *days* (end of expedition) septa exposed for 10 mm. dead zooxanthellae still extruded; tissues perfect, but almost white. Fixed Bouin	..	115 ,,
C4	11.v.29	*After* 23 *days* tissues very emaciated; retreated to edge of septa, perforated everywhere, zooxanthellae being extruded, all dead and degenerating	3.vi.29	23 ,,

In addition to the above, 16 other *Fungia*, making 20 in all, were experimented upon. Of these, two lived for 2 days, one for 3, two for 4, one for 5, three for 6, four for 7, one for 8, one for 14 and one for 44 days.

D. *Fed in Light.*

No.	Date.	Remarks.	Died.	Period in experiment.
D1	27.xi.28	*After* 79 *days* condition excellent, exactly as when taken from the sea, colour normal deep brown, tissue everywhere intact and no retreat from mouth; tentacles expand at night (see Plate II, fig. 8). Died as result of accidental pollution of water.	18.iii.29	111 days.
D2	27.iii.29	Remained throughout in excellent condition	10.v.29	44 ,,
D3	10.v.29	*After* 74 *days* (end of expedition) was in perfect condition, colour deep brown and tissues everywhere intact	..	74 ,,

The ease with which *Fungia* was kept under experimental conditions when fed in the light (D.), only one out of three specimens used dying a natural death in the course of the experiment, is clearly demonstrated by the results recorded above. Plate II, fig. 8, shows the appearance of D1 after 79 days in the experiment, and shows that it was in every way normal. These results, taken in conjunction with those for series B, show that the experimental conditions and the type of food and frequency of feeding fully met the normal requirements of the corals.

The results of these four series of experiments with *Fungia* indicate that this coral can live almost equally well in light and in darkness if suitably fed (and if conditions of temperature and aëration are normal), but that in the darkness the zooxanthellae die and are expelled, the animal being apparently none the worse. But when starved in either light or darkness the corals, *despite the presence of zooxanthellae*, are much more difficult

to maintain alive. They first of all extrude their zooxanthellae in very large numbers; many of these are dead, but some are apparently still alive. This confirms the results of experiments, recorded in Paper IV of this series, on the effect of high temperatures on corals, which indicated that as soon as the metabolism of corals is lowered—in that case by high temperature, in this case by starvation—the zooxanthellae are unable to obtain the necessary supplies of carbon dioxide, nitrogen, phosphorus, etc., and that, as a result, a proportion of them is expelled from the tissues. In many cases this apparently takes place *before* the zooxanthellae are dead.

The second response of the *Fungia* to starvation in both light and darkness, was invariably to reduce their tissues, which was already apparent after 9 days (C2), and became strikingly obvious after longer periods of starvation, as shown in Plate II, figs. 4 and 5, and in Text-fig. 3. There was absolutely no evidence, therefore, from the external appearance of these corals to indicate that they obtained any nutriment whatever from their contained zooxanthellae. The experiments of Vaughan (1930) were confirmed in their entirety.

(ii) *Goniastrea* sp.

Experiments with species of this genus were rendered difficult owing to the impossibility of thoroughly cleansing the bases of the small colonies employed after they had been broken off. Decomposition almost invariably set in at the base, the water was polluted and the coral died, usually after about a week, and, with two notable exceptions, always within a month. Attempts to overcome this difficulty by covering the base of the colonies with cement were unsuccessful. Nevertheless, two colonies gave exceptionally clear and satisfactory results, one in series A and the other in series B. These were both perfectly healthy at the end of 70 and 160 days respectively, when they were removed and fixed in Bouin's fluid. This section will deal with observations on these two colonies.

A. *Starved in Light.*

A1. Placed in experiment on 5th October, 1928; removed and fixed on 14th December, 1928, after 70 days.

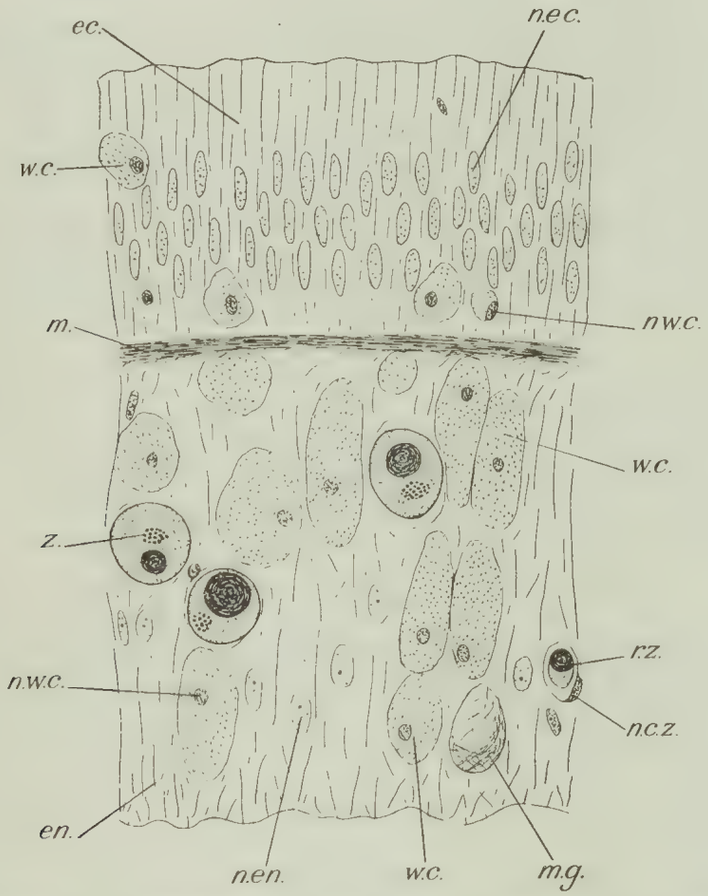
Remarks.—After 52 days this colony was almost pure white.

There was a ring of dark material around the base of the coral at the base of the jar, and also around the periphery of each polyp. Microscopical examination showed that this was composed of zooxanthellae with a little binding mucus. The majority of these zooxanthellae were dead and degenerating; a few only showed the normal structure.

After 70 days the tissues were practically colourless, with occasional dark streaks and patches showing the presence of a few zooxanthellae. Over about one-third of the surface the coenosarc had gone, apparently as a result of dedifferentiation as in *Fungia*. Infection of the skeleton with blue-green algae had caused these exposed regions to become green in colour. This was clearly *not* the cause of the retreat of the coenosarc, because the regions from which the coenosarc had most recently retreated were still white. At the bottom of the jar there were many dark brown masses of zooxanthellae, some of considerable size, *e.g.* several millimetres in length.

Sections subsequently cut of this material confirmed these macroscopical observations. The mesenterial filaments contained a few zooxanthellae in the " absorptive " zone; the

majority of these appeared to be dead and were clearly in process of ejection. The condition in the coenosarc is of especial interest, and is illustrated in Text-fig. 4. A few zooxanthellae (*z.*), some of them greatly reduced in size (*r.z.*), but few showing any clear indication of degeneration, are present in the endoderm (*en.*). They are all contained within cells, the nucleus of one of which (*n.c.z.*) is clearly shown. Of especial interest is the great abundance of wandering cells (*w.c.*) with granular contents and characteristic



TEXT-FIG. 4.—*Goniastrea* sp., section through the coenosarc of specimen A1, after it had been starved in the light for 70 days. Fixed Bouin, stained safranin and light green. × 1200. *ec.*, ectoderm; *en.*, endoderm; *m.*, mesogloea; *m.g.*, mucus-gland; *n.c.z.*, nucleus of cell containing zooxanthella; *n.ec.*, nucleus of ectoderm cell; *n.en.*, nucleus of endoderm cell; *n.w.c.*, nucleus of wandering cell; *r.z.*, reduced zooxanthella; *w.c.*, wandering cell; *z.*, zooxanthella.

small, round, darkly-staining nuclei (*n.w.c.*). These are also conspicuous in the ectoderm (*ec.*). The condition, therefore, resembles in every way that which was observed after long subjection of similar corals (*Favia*) to darkness, as described in Paper IV and illustrated there in Text-fig. 15. The tissues appear healthy in spite of the long period of starvation, although the exceptional thinness of the mesogloea (*m.*) may be the result of these unfavourable conditions. Important evidence is again forthcoming to show that whatever be the unfavourable conditions to which the corals are exposed, the effect of the consequent lowering of their metabolic activities is the expulsion of a large

proportion of their contained zooxanthellae and an increase in the number of wandering cells. But there is still no evidence that the zooxanthellae are digested by the corals.

B. *Fed in Darkness.*

B1. Placed in experiment on 15th January, 1929; removed and fixed on 24th June, 1929, after 160 days.

Remarks.—After 36 *days* zooxanthellae were being extruded.

After 63 *days* the colony was distinctly paler and zooxanthellae were being extruded in increasing numbers.

After 133 *days* the colony was almost pure white and there was no sign of zooxanthellae being extruded; practically all had, apparently, already been ejected.

After 150 *days* the colony was pure white, but the tissues were intact and healthy and no zooxanthellae were extruded. The excretion of phosphorus by this coral was investigated, the results being given and commented on in Section 4 of this paper.

The results of this experiment confirm those with *Fungia*, and show that corals confined in the dark, but adequately fed, can live for indefinite periods with no other change than a loss of their contained zooxanthellae.

(iii) *Psammocora gonagra.*

This coral lived extraordinarily well under all four sets of experimental conditions, and gave results of a most convincing character. The original colonies placed in the experiment in all cases survived for the full period.

A. *Starved in Light.*

A1. Placed in experiment on 8th November, 1928; removed on 24th June, 1929, after 228 days.

Remarks.—After 22 *days* the colour was not obviously changed, but several masses of zooxanthellae were found at the bottom of the jar; the majority of these were dead.

After 92 *days* the tissue (as seen under the binocular dissecting microscope) was withdrawn, in many places exposing white patches of skeleton. Coenosarc intact within grooves between ridges of skeleton and here still contained many zooxanthellae. Zooxanthellae being extruded in very large numbers. These were collected from the base of the jar and a number tested for the presence of the cellulose wall by the usual tests (see Paper IV). In all cases, though the algae were dead *the cellulose wall was found to be intact.* Portions fixed in Flemming and Bouin.

After 166 *days* coenosarc still further reduced and zooxanthellae still being discharged in large numbers. Portions fixed as before.

After 218 *days* coenosarc practically colourless and still further reduced. Slight brown tinge only in polyps. Phosphorus excretion examined (see Section 4).

After 228 *days* remainder fixed as before.

B. *Fed in Darkness.*

B1. Placed in experiment on 8th November, 1928; removed on 24th June, 1929, after 228 days.

Remarks.—After 22 *days* tissues apparently unchanged in colour; a few zooxanthellae found extruded.

After 92 *days* the tissues generally decidedly lighter than originally, but a universal paling, dark areas not being restricted to grooves because the coenosarc is everywhere intact. Zooxanthellae extruded in moderate numbers. Portions fixed as before.

After 166 *days* coenosarc everywhere intact but now almost colourless, although no longer any great discharge of zooxanthellae. Portions fixed as before.

After 218 *days* coenosarc pure white throughout and everywhere intact. Zooxanthellae no longer being extruded in any quantity. Phosphorus excretion examined.

After 228 *days* remainder fixed as before.

C. *Starved in Darkness.*

C1. Placed in experiment on 8th November, 1928; removed on 24th June, 1929, after 228 days.

Remarks. After 22 *days* no obvious change, but zooxanthellae being extruded in small numbers.

After 92 *days* coenosarc paling, but with many light patches due to withdrawal of tissues from skeleton as in series A. Zooxanthellae being extruded in very great numbers; masses of them bound in mucus seen lying on the surface of the coenosarc. Portions fixed as before.

After 166 *days* coenosarc extremely pale, almost pure white, but not withdrawn from skeleton to same extent as in series A, probably about three-quarters intact. Zooxanthellae still being extruded. Portions fixed as before.

After 218 *days* coenosarc practically colourless and further reduced. As in series A, slight brown colour only in polyps. Phosphorus excretion examined.

After 228 *days* remainder fixed as before.

D. *Fed in Light.*

D1. Placed in experiment on 8th November, 1928; removed on 24th June, 1929, after 228 days.

Remarks. After 22 *days* colony in every way normal and no zooxanthellae being extruded.

After 92 *days* colony retains normal brown colour with no light patches and is everywhere intact. No zooxanthellae extruded. Portions fixed as before.

After 166 *days* colour a little paler than normal, the result, presumably, of rather more shady conditions than those to which normally exposed. Coenosarc everywhere intact and no zooxanthellae extruded. Portions fixed as before.

After 218 *days* still dark brown except for slightly paler areas near tips of branches. No zooxanthellae observed to be extruded. Phosphorus excretion examined.

After 228 *days* remainder fixed as before.

Sections were subsequently prepared of much of the fixed material, and results obtained confirm and extend the observations recorded above. Sections of A1 fixed in

Flemming's fluid 166 days after it had been placed in the experiment showed the presence of a few zooxanthellae in the endoderm of the coenosarc and other superficial regions. These were reduced in size, but there was no definite evidence of degeneration *in situ*. As a result of starvation of the coral, there were no oil-droplets within the zooxanthellae. In the mesenterial filaments, as shown in Plate III, fig. 9, there were many zooxanthellae in the " absorptive " zone (*a.z.*). Of those shown in the figure, two appear normal and healthy (*z.*), although somewhat reduced in size, but the third (*z.d.*), though still spherical and with the cellulose wall intact, has little internal structure and is clearly dead. Here again no oil-droplets appear within the zooxanthellae, nor is there any fat in the tissues, although there are a number of granules (*g.*) which stain darkly with safranin. *There is no evidence whatever that the zooxanthellae are being digested as a result of the starvation of the coral in which they live.*

Sections cut of B1 fixed in Flemming's fluid after 92 days showed many of the zooxanthellae which still remained in the endoderm of the coenosarc and elsewhere with clear signs of degeneration *in situ*. This is indicated in Plate III, fig. 10, where four zooxanthellae (*z.d.*) are shown lying within cells and all of them in various stages of degeneration. They are of various shapes, but in all cases *the cellulose wall is intact*, and there is no evidence of any transfer of material from the zooxanthellae to the substance of the endoderm. There is a notable absence of oil-droplets within the zooxanthellae, the result of deprivation of light. As in *Goniastrea*, already commented upon and figured, there are wandering cells (*w.c.*) within the endoderm. Similar degenerating zooxanthellae are present in the " absorptive " zone of the mesenterial filaments in process of ejection from the tissues. Here, again, there is no evidence of any digestion of the zooxanthellae, although since the coral was adequately fed, this would not be considered necessary by those who uphold the view that corals feed on their zooxanthellae only during periods of starvation. It is clear that when exposed to complete darkness the zooxanthellae frequently die *in situ* owing to their inability to synthesize starch, and are then extruded. Similar results were obtained from material fixed after 166 days.

Section of C1 fixed in Flemming's fluid after 92 and 166 days showed that the conditions are in no way different from those recorded for B1. Apparently, therefore, the fact that the coral is deprived of food does not, under these conditions, make any difference to the zooxanthellae. This is in no way surprising, because the quantity of material excreted by the coral, carbon dioxide, nitrogen, phosphorus, etc., must be immaterial to the zooxanthellae, which, owing to the absence of light, cannot synthesize carbohydrate. Here, again, there is absolutely nothing in the sections to indicate that the zooxanthellae are digested by the coral.

In the control coral, D1, sections of material fixed in Flemming's fluid after 166 days showed that there were numerous healthy zooxanthellae within the endoderm of the coenosarc, disc, etc. Very few zooxanthellae were present in the " absorptive " zone of the mesenterial filaments. In every respect the conditions were identical with those observed in sections of *Psammocora* taken direct from the sea and fixed in Flemming's fluid, except that the zooxanthellae were a little less numerous in the coenosarc, etc., owing to the more shady conditions under which the experimental coral was kept.

The results of these observations on *Psammocora* agree with those already recorded for *Fungia* and *Goniastrea*, the examination of sections confirming in every respect the impression gained from the external appearance of the different experimental

corals. *Psammocora* fed either in light or in darkness remains in a healthy condition and maintains its tissues, only losing its zooxanthellae in the dark. When starved in light or darkness the tissues are quickly reduced, and zooxanthellae, many dead but some still living, are ejected in great numbers. There is still no evidence of the digestion of the zooxanthellae or of the transference of any material from them to the tissues of the coral. This negative evidence is provided equally by the general appearance of the experimental corals and by their histological condition. Further experimental evidence will be provided in Section 4 of this paper, which deals with work on oxygen exchange between these corals and surrounding water in both light and darkness, and with phosphorus excretion by them.

(iv) *Galaxea fascicularis.*

Experiments with this coral were not so successful as with *Psammocora*; nevertheless the results obtained are valuable because they confirm those already recorded.

A. *Starved in Light.*

A1. Placed in experiment on 9th November, 1928; removed on 11th December, 1928, after 32 days.

Remarks.—After 19 *days* tissues healthy but withdrawn from outer wall of column and from exsert septa; remaining disc tissue between projecting septa very dark brown. Examination of macerated tissues revealed that there were many zooxanthellae, only about 10% of which showed signs of degeneration. In the mesenterial filaments there were considerable numbers of zooxanthellae in the "absorptive" zone, 50% of which were degenerating.

After 32 *days* almost no tissue could be seen and with four exceptions the polyps appeared to be dead, but further examination showed that in all cases the tissues within the polyps were present and healthy. Great numbers of zooxanthellae had been extruded. Examination of the mesenterial filaments revealed the presence of exceptionally large numbers of zooxanthellae, the majority of which showed little evidence of disintegration. Remainder of colony fixed in Bouin.

In addition to A1, five other colonies of *Galaxea* were starved in the light, but all of them died within 10 days.

B. *Fed in Darkness.*

B1. Placed in experiment on 9th November, 1928; removed on 12th December, 1928, after 33 days.

Remarks.—After 19 *days* coenosarc practically intact, although a few perforations, decidedly lighter in colour. Disc intact but pale and tentacles withdrawn. Examination of the tissues showed that the zooxanthellae were still abundant in the superficial endoderm and all appeared still healthy, but that of the exceptionally numerous zooxanthellae in the mesenterial filaments about half were degenerating.

After 33 *days* the colony was still very healthy, although the coenosarc was paler owing to further loss of zooxanthellae. Those in the superficial endoderm, though less numerous, appeared healthy, and there were fewer than before in the "absorptive" zone of the mesenterial filaments. Remainder of colony fixed in Bouin.

B2. Placed in experiment on 12th December, 1928; removed on 28th March, 1929, after 106 days.

Remarks.—After 70 *days* general appearance resembled that of *Galaxea* taken from a depth of 9 fathoms (see Paper IV) owing to great diminution in numbers of zooxanthellae in the tissues. Those extruded all found to be dead and degenerating.

After 106 *days* a few strands of colourless tissue only remained on the outer surface of the polyps, each containing a few partially disintegrating zooxanthellae. Tissue of disc and tentacles colourless, but on examination under the high powers of the microscope a few dead zooxanthellae were seen. Zooxanthellae most numerous in tissue clothing exsert septa, which was brown in colour owing to the presence of masses of algae which, with one or two exceptions, were all dead. The mesenterial filaments were very pale and contained sparse zooxanthellae, all of them dead and lying within the "absorptive" zone.

C. *Starved in Darkness.*

C1. Placed in experiment on 9th November, 1928; removed on 12th December, 1928, after 33 days.

Remarks.—After 19 *days* general condition of colony resembled that of B1, but two polyps dead. In the coenosarc and disc about 10% of the zooxanthellae were dead; in the mesenterial filaments they were more abundant than in B1 and about 75% were clearly degenerating.

After 33 *days* the colony was dead, the external tissue having disintegrated and gone, and the interior of the polyps being occupied by a mass of disintegrating zooxanthellae mixed with mucus.

In addition to C1, five other colonies of *Galaxea* were fed in the dark, but all of them died within 9 days.

D. *Fed in Light.*

D1. Placed in experiment on 9th November, 1928; removed on 9th February, 1929, after 92 days.

Remarks.—After 19 *days* condition perfect and colour normal. Examination of the tissues showed that zooxanthellae were as abundant as usual in the superficial endoderm, and not more than one in a thousand showed signs of degeneration. In the mesenterial filaments there were very few as compared with A1. In a typical case 11 were present under the field of the microscope as compared with 100 in A1. Of these 11, 6 were clearly degenerate.

After 32 *days* the colony was still perfectly healthy, the polyps expanding frequently by day as well as by night. There were abundant zooxanthellae in the superficial endoderm, all apparently healthy. There were very few zooxanthellae in the mesenterial filaments and of these some were degenerate.

After 92 *days* all the polyps remained perfectly healthy, although after this long period the coenosarc had become reduced in many places and the skeleton exposed. But the tissues had still the usual brown colour and the zooxanthellae were abundant and healthy. Remainder of colony fixed in Bouin.

The effect of starvation of *Galaxea* in both light and darkness was as immediate as in the other corals already reported upon. In this case, however, the coral did not, apparently, feed so readily under experimental conditions, especially in the dark. In

consequence of this the fed corals, although they lived much longer and remained in much better condition than the starved corals, did eventually lose a portion of their tissues, although the zooxanthellae in the control coral, D1, remained abundant and healthy to the last.

(v) *Cyphastrea chalcidicum.*

Small portions were broken off the large brown colonies of this coral, and these successfully survived exposure to experimental conditions.

A. *Starved in Light.*

A1. Placed in experiment on 4th February, 1929 ; removed on 19th April, 1929, after 74 days.

Remarks.—After 74 *days* the coenosarc had largely disappeared, and such tissue as remained was much paler than normal. Zooxanthellae were extruded in large numbers. Fixed in Bouin.

Sections subsequently prepared of this material showed that there were still a certain number of apparently healthy zooxanthellae in the superficial endoderm, though much less abundant than in the control coral, D1. In the " absorptive " zone of the mesenterial filaments there were large numbers of zooxanthellae in process of extrusion, many of them being degenerate.

B. *Fed in Darkness.*

B1. Placed in experiment on 4th February, 1929 ; found dead owing to decay at base of skeleton on 10th April, 1929, after 65 days.

B2. Placed in experiment on 11th April, 1929 ; removed on 24th June, 1929, after 74 days.

Remarks. -After 74 *days* the coenosarc was very pale, the polyps being a deeper brown, but the tissues were everywhere intact. Zooxanthellae were extruded. Fixed in Bouin.

Later examination of sections showed that zooxanthellae were still comparatively numerous in the superficial endoderm, but that many of them were degenerate. Similar degenerate zooxanthellae were being extruded from the coral by way of the " absorptive " zone of the mesenterial filaments.

C. *Starved in Darkness.*

C1. Placed in experiment on 16th February, 1929 ; removed on 19th April, 1929, after 62 days.

Remarks. -After 62 *days* the appearance of this coral closely resembled that of B2, except that the coenosarc had disappeared in many places. Zooxanthellae were extruded. Fixed in Bouin.

The appearance of the tissues in sections resembled in every way that of B2.

D. *Fed in Light.*

D1. Placed in experiment on 4th February, 1929 ; removed on 19th April, 1929, after 74 days.

Remarks. *After 74 days* the coenosarc was intact and the colony in every way normal except for a very slight paling. Zooxanthellae were not extruded to any noticeable degree. Fixed in Bouin.

In sections the zooxanthellae were revealed as being very numerous, especially in the superficial endoderm, and being everywhere healthy. There were very few in the "absorptive" zone of the mesenterial filaments and only a few of these were degenerate.

The results recorded above demonstrate clearly that the effects of these various experimental conditions upon *Cyphastrea* are identical with those on the other corals. The starved corals show no sign whatever of obtaining nourishment from their zooxanthellae.

(vi) *Lobophyllia corymbosa.*

Single polyps only of this coral were used in each experimental jar.

A. *Starved in Light.*

A1. Placed in experiment on 1st February, 1929; removed on 18th April, 1929, after 76 days.

Remarks. *After 69 days* immense numbers of zooxanthellae were extruded so that a thick, brown deposit was formed on the bottom of the jar. The tissue was very thin and very pale, and the edge-zone had retreated almost to the edge of the disc, which was itself intact.

After 76 days the edge-zone had retreated so far that it had disappeared and only the summit of the calyx was covered with tissue, the disc being intact, but white and semi-transparent.

B. *Fed in Darkness.*

B1. Placed in experiment on 1st February, 1929; removed on 18th April, 1929, after 76 days.

Remarks. *After 46 days* the tissues were already very pale, although showing no signs of diminution. Great numbers of zooxanthellae were extruded.

After 76 days the tissues were white and semi-transparent and the edge-zone had retreated a little, indicating that the coral had not fed well under these conditions. Zooxanthellae were still being extruded.

C. *Starved in Darkness.*

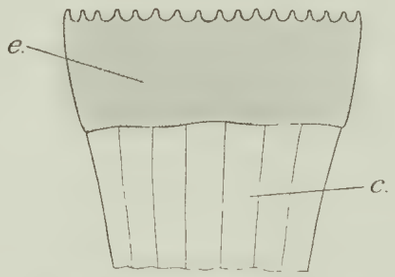
Five polyps were successively tried, but all died within seven days, which confirms the impression already gained from B1 that *Lobophyllia* does not live well in complete darkness.

D. *Fed in Light.*

D1. Placed in experiment on 26th March, 1929; removed on 23rd July, 1929, after 119 days.

Remarks. *After 119 days* the tissues, including the edge-zone, were everywhere intact and healthy, although a shade paler than normal. No zooxanthellae were extruded.

Lobophyllia corymbosa, although it does not live well in darkness, shows the effect of starvation in the light almost immediately by the extrusion of immense numbers of zooxanthellae and by the reduction of its tissues. The edge-zone, being unattached at its lower extremity (see Text-fig. 5), is free to retreat without rupturing, and this provides a clear demonstration of the effect of starvation. The large and fleshy edge-zone of a polyp, the extent of which is shown in Text-fig. 5, *e.*, had entirely disappeared after 76 days' starvation, although that of a similar polyp fed in the light was intact after 119 days. It is interesting to compare the reduction of the tissues in *Lobophyllia* with their reduction in *Fungia*, where they are unattached only around the mouth. But in both cases the inability of the corals to obtain nourishment from the zooxanthellae is equally clear.



TEXT-FIG. 5.—*Lobophyllia corymbosa*, outline of a single polyp showing extent of edge-zone tissue. Nat. size. *c.*, coenosteum (bare skeleton); *e.*, edge-zone.

(vii) *Pocillopora bulbosa.*

Although, as already stated, adult colonies of *Pocillopora*—either small pieces broken off large colonies or very small complete colonies—did not survive in the experiment, successful results were obtained with newly-settled colonies. Planulae were collected in large numbers and placed in jars such as were used in the experiment. After a few days the majority of the planulae settled on the sides and bottom of the jars and these were then placed in the experiment. Other planulae were induced to settle upon glass slides and then these were suspended in jars in the experiment. This proved the more suitable method, because the slides could be removed from time to time and the young colonies carefully examined under the binocular dissecting microscope.

A. *Starved in Light.*

A1. Placed in experiment on 23rd February, 1929; removed and fixed in Bouin and Flemming on 19th March, 1929, after 24 days.

Remarks.—After 24 days these corals were still healthy but much paler than usual, and there were no buds around the original polyps.

Sections cut of material fixed in Flemming's fluid showed that zooxanthellae remained in moderate numbers in the endoderm generally. These, as shown in Plate III, fig. 11, were all healthy and still contained some oil-droplets (*o.*), especially around the assimilation product. There were fat-globules (*f.*) in the endoderm, but not the slightest evidence to show that these might have come from the zooxanthellae. It is worthy of note that

two of the zooxanthellae (*z.*) shown in the figure, those on the right-hand side, both appear to be contained within ordinary endoderm cells, judging by the nature of the nucleus (*n.c.z.*). This is not in agreement with the suggestion advanced in Paper IV that the zooxanthellae may *always* be contained within wandering cells. It also indicates that the endoderm is *not* a syncytium.

Sections through the mesenterial filaments showed that the zooxanthellae were numerous within them and that they were being ejected from the "absorptive" zone. The typical condition is shown in Plate III, fig. 12. Two (*z.d.*) out of the three zooxanthellae shown in the figure are degenerating, but these, together with the normal one (*z.*), still retain their cellulose wall and spherical shape. Although there is abundant fat (*f.*) in this region as well as granules (*g.*), which stain red with saffranin, there is no evidence that the zooxanthellae are digested, or that there is any passage of material from them into the tissues of the coral.

B. *Fed in Darkness.*

B1. Placed in experiment on 24th December, 1928; removed on 19th March, 1929, after 77 days.

Remarks.— *After* 46 *days* polyps were rather smaller than those of control series, D, but growing and budding in the same way. *No sign of any brown colour*, the expanded tentacles and tissues generally being completely transparent.

After 77 *days* all were dead with the exception of one polyp, which appeared the same as before.

B2. Placed in experiment on 23rd February, 1929; removed and fixed in Flemming on 13th April, 1929, after 49 days.

Remarks.—*After* 24 *days* much paler than normal but healthy. A few polyps were fixed in Flemming's fluid, and sections later showed that there were comparatively few zooxanthellae in the superficial endoderm, but those remaining appeared healthy and still possessed oil-droplets. Only a very few were seen in the mesenterial filaments, but all of these were degenerate, though still spherical.

After 49 *days* the polyps had grown and budded to the same extent as those which had been for exactly the same period in the light (D2). The tentacles expanded readily, and when examined under the binocular microscope were seen to be completely colourless and transparent. There were no dead colonies, and all were fixed in Bouin or Flemming's fluids.

The examination subsequently of sections showed that only a very few solitary degenerate zooxanthellae remained in the superficial endoderm, and that they were most abundant within the "absorptive" zone of the mesenterial filaments. Here, also, they were invariably degenerating, and were in process of expulsion from the tissues.

C. *Starved in Darkness.*

C1. Placed in experiment on 23rd February, 1929; removed on 13th April, 1929, after 49 days.

C2. Placed in experiment on 2nd March, 1929; removed on 19th March, 1929, after 17 days.

Remarks.—In both cases all polyps were dead at the end of these periods.

D. *Fed in Light.*

D1. Placed in experiment on 24th December, 1928; removed on 19th March, 1929; after 77 days.

Remarks. *After* 46 *days* the polyps were normal and budding and possessed well-developed, brown tentacles.

After 77 *days* still perfectly healthy and normal in colour and appearance.

D2. Placed in experiment on 23rd February, 1929, removed on 13th April, 1929, after 49 days.

Remarks. *After* 24 *days* these polyps were healthy and dividing and of the normal brown colour. Certain of them were fixed in Flemming's fluid, and subsequent examination showed that there were abundant zooxanthellae, with but few exceptions healthy, in the superficial endoderm, and a very few, and these usually apparently healthy, in the " absorptive " zone of the mesenterial filaments.

After 49 *days* the polyps were well developed, with in most cases numerous young polyps in a ring around the original one. Zooxanthellae were numerous and healthy, as was revealed by the brown colour of the expanded tentacles and by the ceonosarc generally. None was dead.

These experiments on newly-settled *Pocillopora bulbosa* are of especial interest, in that they demonstrate that young coral colonies can grow and develop in the absence of light and so of the zooxanthellae (series B). Starved colonies, whether the zooxanthellae were present, as in the light (A), or absent, as in the darkness (C), failed to grow, and those in dark soon died. Equally in the young as in the mature colonies, therefore, the presence of zooxanthellae is of no assistance in nutrition.

(viii) *Dendrophyllia nigrescens.*

Single large polyps of this coral were placed in the four series of experimental jars. This coral, as already shown in Paper IV of this series, contains no zooxanthellae, and it was placed in the experiment solely with the aim of discovering the reactions of such a coral to these experimental conditions, and thereby affording a control for the other corals, which all contained zooxanthellae.

A. *Starved in Light.*

A1. Placed in experiment on 24th March, 1929; removed on 23rd July, 1929, after 121 days.

Remarks.—After 82 *days* the coral was still alive but the coenosarc was considerably reduced, and the polyp never observed expanded, even at night.

After 121 *days* coenosarc still further reduced but polyp still alive.

B. *Fed in Darkness.*

B1. Placed in experiment on 24th March, 1929; removed on 23rd July, 1929, after 121 days.

Remarks. *–After* 82 *days* the tissues were normal in every way and the polyp was continually expanded.

After 121 *days* the polyp continued perfectly healthy and expanded.

C. *Starved in Darkness.*

C1. Placed in experiment on 24th March, 1929; removed on 23rd July, 1929, after 121 days.

Remarks.- After 82 *days* the coenosarc was greatly reduced and the polyp remained permanently contracted.

After 121 *days* the coenosarc was almost completely gone, but the polyp still remained alive, although contracted.

D. *Fed in Light.*

D1. Placed in experiment on 24th March, 1929; removed on 23th July, 1929, after 121 days.

Remarks. -After 82 *days* the polyp was normal in every respect and the coenosarc was intact.

After 121 *days* the polyp was still healthy and expanded at night, although a little of the coenosarc had gone.

Although at the end of the long period of 121 days both of the fed corals, B and D, showed a slight reduction of the tissue of the coenosarc, they were otherwise perfectly healthy and the polyps expanded freely. On the other hand, the starved corals, A and C, showed the effects of deprivation of food in the great reduction of their tissues and the permanent contraction of their polyps. *Dendrophyllia*, therefore, is affected by the various experimental conditions in exactly the same way as the other corals which contain zooxanthellae, except, of course, that there are no contained zooxanthellae for it to expel. Work on this coral thus provides proof of the adequacy of the experimental conditions, and further confirmation of the fact that corals with zooxanthellae cannot obtain nourishment from them, because they are affected by starvation in exactly the same manner and to the same extent as corals, such as *Dendrophyllia*, which have no zooxanthellae.

4. OXYGEN EXCHANGE AND PHOSPHORUS EXCRETION IN EXPERIMENTAL CORALS.

(*a*) Oxygen Exchange.

After they had been for 137 days in the experiment, the four specimens of *Psammocora gonagra*, A1, B1, C1 and D1, were removed for one day, and the oxygen exchange in light and in darkness between each of them and definite volumes of sea-water was determined. They were placed in 7-lb. glass jars with screw tops, which were filled with water and the tops secured under water in large buckets, so that no air was present in the sealed jars. Full details of the methods employed will be given in Paper VI of this series. Each of the jars had a capacity of approximately 2800 c.c.

The temperature and oxygen content of the water which had been taken from the open waters of the anchorage was first determined, then the jars were filled. They were then placed under even conditions of temperature in the light for nine hours, when they were opened, the temperature taken and samples of water drawn off for oxygen determinations. The jars were then refilled, the different specimens of *Psammocora* remaining in their original jars, and the same process was repeated with the jars in complete darkness under inverted buckets in the sand beneath the aquarium.

The results of the two sets of experiments are given in Table I. In Table II the percentage changes of the oxygen content of the water in light and in darkness and the difference between these is given. The difference in terms of unit volume of the corals (1 c.c.) is also given, and this provides the best indication of the differences in oxygen utilization and production by the corals consequent respectively on their metabolic state (the result of starvation or feeding) and on the numbers of zooxanthellae contained within them.

TABLE I.—*Oxygen Exchange in* Psammocora gonagra *after* 137 *Days in Experiment. Oxygen given in Terms of c.c. per Litre.*

No.	Treatment.	Nine hours in light.				Nine hours in darkness.			
		Initial temperature.	Final temperature.	Initial oxygen.	Final oxygen.	Initial temperature.	Final temperature.	Initial oxygen.	Final oxygen.
A1	Starved light	28·0° C.	28·9° C.	4·54	4·26	27·0° C.	26·2° C.	4·49	3·92
B1	Fed dark	,,	,,	,,	4·07	,,	,,	,,	3·87
C1	Starved dark	,,	,,	,,	4·07	,,	,,	,,	3·89
D1	Fed light	,,	,,	,,	4·56	,,	,,	,,	4·07

TABLE II.—*Percentage Changes in Oxygen Content of Sea Water in Light and in Darkness, also Difference in Terms of Unit Volume* (1 *c.c.*) *of Corals.*

No.	Volume.	Percentage change in oxygen content.		Total difference.	Difference in terms of unit volume.
		Light.	Darkness.		
A1	9 c.c.	93·84	87·64	6·20	0·69
B1	9 c.c.	89·64	86·19	3·45	0·383
C1	7·5 c.c.	89·64	86·64	3·0	0·40
D1	6 c.c.	100·44	90·64	9·8	1·09

An analysis of the above results shows that the coral fed in the light, D1, alone possessed sufficient zooxanthellae for the production of oxygen in the light by these more than to counterbalance the utilization of oxygen by the coral itself. In other words, this behaved like any normal reef-building coral (full details will be given in Paper VI). Without exception the other three corals utilized more oxygen than was produced by their zooxanthellae, although, as shown by the difference between the fall in oxygen in light and darkness (when the chlorophyll of the zooxanthellae would be unable to function), they all contained some zooxanthellae. The relative numbers of zooxanthellae in the four corals are indicated by the figures in the fourth column of Table II. After D1, most zooxanthellae were contained in the coral starved in the light, A1, which was to be expected, since the zooxanthellae were exposed to light and only limited in numbers by the diminution in the supplies of carbon dioxide, nitrogen, phosphorus, etc., consequent upon

the lowered metabolism of the coral. The two corals which had been kept in darkness, B1 and C1, possessed approximately equal numbers of zooxanthellae, less than 40% of those in D1 and about 57% of those in A1. The resemblance in the content of zooxanthellae in these two corals indicates that the plants were affected primarily by the same disadvantageous circumstance—absence of light—and that there was no additional lowering of the numbers of zooxanthellae in the starved coral, C1, as a result of digestion of the zooxanthellae by the coral. These results, therefore, afford further confirmation of the conclusion already arrived at, that the corals cannot digest zooxanthellae under any circumstances.

(*b*) Phosphorus Excretion.

As already stated in the remarks on the experiments with *Psammocora*, the phosphorus excretion in these corals, A1–D1, was determined after they had been in the experiment for 218 days. The opportunity was also taken to determine the phosphorus excretion in *Goniastrea*, B1, which had been fed in darkness for 150 days. The methods employed were identical with those described in Paper IV. Table III summarizes the results of the determinations. The figures given are of phosphorus in terms of milligrammes per cubic metre, and no allowance has been made for the reduction of the water by the removal of samples, since comparative results only were required. The results in terms of unit volume are also given for the final set of readings, allowance being made for the original concentration of phosphorus in the water.

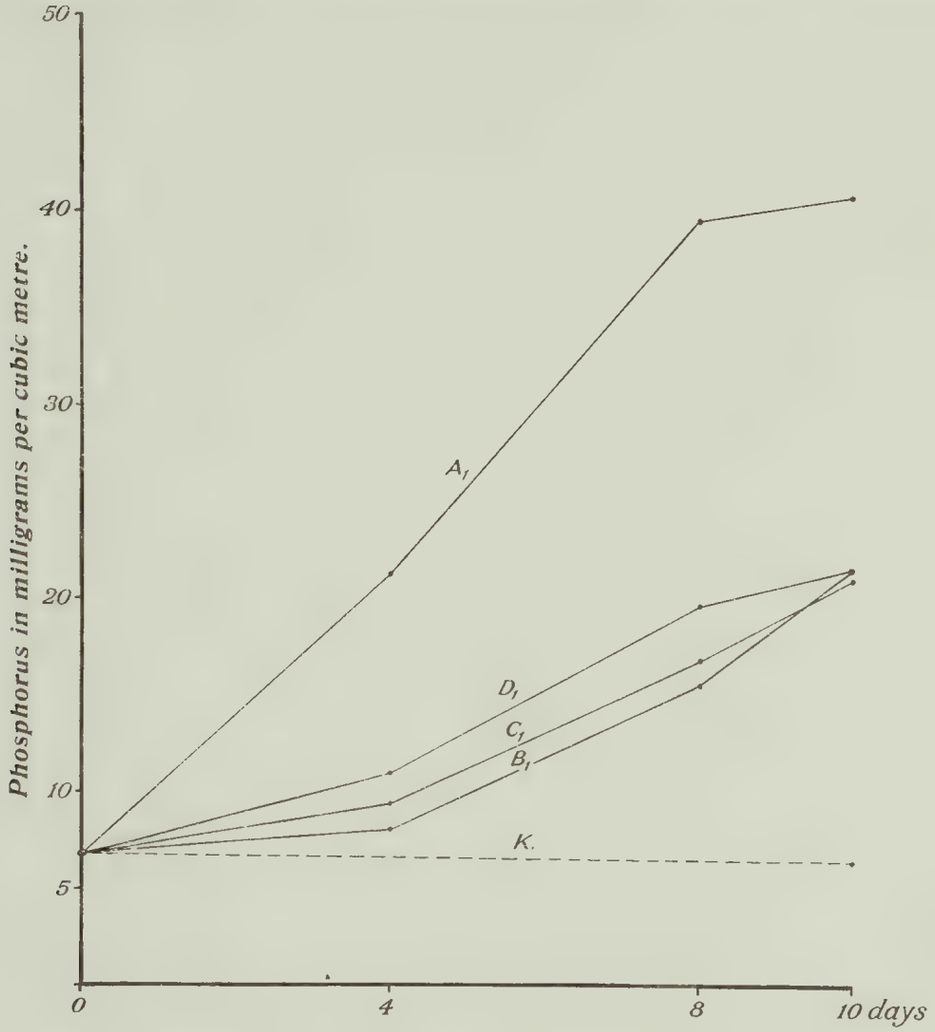
Table III.—*Phosphorus Excretion by* Psammocora gonagra *and* Goniastrea *sp. after Exposure to Experimental Conditions.*

Coral.	Volume.	Treatment.	Period.	Phosphorus in water:				Production per unit volume.
				Initial.	4 days.	8 days.	10 days.	
Psammocora A1	6 c.c.	Starved light	218 days	6·77	21·19	39·50	40·34	12·365
,, B1	6 ,,	Fed dark	218 ,,	6·77	7·97	15·47	21·41	9·21
,, C1	5 ,,	Starved dark	218 ,,	6·77	9·35	16·74	20·93	9·602
,, D1	4·5 ,,	Fed light	218 ,,	6·77	10·93	19·58	21·41	10·023
Goniastrea B1	..	Fed dark	150 ,,	6·77	7·95	17·13	23·82	..
Control	..	..	..	6·77	..	..	6·38	..

It was shown in Paper IV of this series that the phosphorus excreted by corals containing zooxanthellae is intercepted, completely (as in *Favia* and *Porites*, see Table II, Paper IV) or in part, by the zooxanthellae. There is a large and continuous excretion of phosphorus by corals, such as *Dendrophyllia*, which possess no zooxanthellae, and by corals which have lost their zooxanthellae following long exposure to darkness in the light-tight box on the reef flat (see Table V, Paper IV). The figures given in the above Table for *Goniastrea* further confirm these findings.

The results summarized in Table III and shown graphically (for *Psammocora* only) in Text-fig. 6 agree entirely with these previous findings. The greatest excretion of

phosphorus was produced by the coral A1, which was starved in the light, and where there were many fewer zooxanthellae than in the control coral, D1, which was fed, and where the excretion of phosphorus was much lower. An additional confirmation, if such be needed, is thus afforded of the reduction of zooxanthellae as a result of starvation. The figures for the two coral kept in the dark are very similar to each other, and also to that for the control coral. It was shown in the oxygen experiment that the numbers of zooxanthellae in these corals was very similar, and it would be expected, therefore, that the



TEXT-FIG. 6.—Graph showing increase in phosphorus content of water containing four specimens of *Psammocora gonagra* after they had been under experimental conditions for 218 days. See Table III. A1, starved light; B1, fed dark; C1, starved dark; D1, fed light; *K.*, control.

higher metabolism of the fed coral, B1, would be shown by an increased excretion of phosphorus. It may be that the zooxanthellae in the fed coral were in somewhat better condition than in the starved coral (sections indicate that this may be so), and so would intercept more phosphorus. But the volumes of the corals were too small for much weight to be placed on small differences. The definitely greater production of phosphorus by the light-starved coral (which, judging by the oxygen results, had many more healthy zooxanthellae in its tissues) would seem to indicate that these corals thrive much

better in light than in *complete* darkness. This confirms the impression already gained from the difficulty of maintaining starved corals of any genus in the dark.

5. DISCUSSION.

The results of the experiments recorded in this paper reveal, in the first place, that the experimental conditions were satisfactory. The most important results were obtained, as was expected, with series A and C—corals starved respectively in light and in darkness. Their most striking response to starvation was *the reduction in the bulk of their tissues*. In the case of *Fungia* this took the form of a great diminution in the tissue of the disc, in *Lobophyllia* of a reduction and final complete disappearance in the edge-zone, in *Goniastrea*, *Psammocora*, *Galaxea* and *Cyphastrea* of a general reduction of the coenosarc. The response of *Dendrophyllia* was identical with that of these last four corals. There was also a greater mortality in series A and C than in the others where food was provided. This was demonstrated particularly clearly in the case of *Fungia*, *Galaxea* and *Pocillopora*. On the other hand, the tissues of corals fed in the light or in the dark were either not reduced at all, or only very slightly after very long periods.

The conclusions that Vaughan (1930) deduced from the results of starvation in *Maendra* are thus abundantly confirmed. Madreporaria when starved quickly show a reduction in the bulk of their tissues, and this is shown equally whether they possess zooxanthellae or they do not.

Even before there is a clear reduction in the tissues, the effects of starvation are shown by the expulsion from the corals of great numbers of zooxanthellae. This lowered content of zooxanthellae was also strikingly demonstrated by the results of the experiments on oxygen exchange in *Psammocora*. They are also quickly expelled from corals fed in the dark owing to their death, which is caused by absence of light. This has already been experimentally demonstrated in Paper IV of this series, where the results of the experiments with corals kept in the light-tight box on the reef-flat were described. But there is a very important difference between the condition of the zooxanthellae expelled by corals starved in the light and those expelled by corals kept in darkness. The former are never all of them dead, whereas the latter are invariably *all* dead. In other words, the zooxanthellae of corals starved in the light do not necessarily die *in situ* (this was indeed never observed in sections), whereas those within corals fed or starved in the dark frequently do so, as the study of sections clearly showed. The zooxanthellae are expelled from corals starved in the light as a result of the *lowered metabolism* of these corals, and the consequent insufficiency of carbon dioxide, nitrogen, phosphorus and other necessary inorganic food substances for plants. It was shown in Paper IV that precisely the same thing happens when the metabolism of the corals is lowered by their exposure to high temperatures. An examination of the zooxanthellae extruded by *Psammocora* A1, after it had been starved for 92 days, showed that these still possessed an intact cellulose wall.

Thus instead of starved corals digesting their zooxanthellae, as Boschma and others (see Introduction) have suggested, their first response to starvation is the expulsion of large numbers of these zooxanthellae, some of them still alive and all largely intact. The study of sections entirely failed to reveal any indication of the digestion of zooxanthellae by the corals in the "absorptive" zone of the mesenterial filaments, whence they are invariably extruded. Boschma's view that the zooxanthellae are first extruded and later

re-ingested and digested is in its very nature improbable, and would require much more exact proof than he has provided. The radical change which this suggested digestion of plant matter would involve has not been considered at all by him. Recent research on the comparative physiology of digestion in invertebrates has shown that animals are as highly specialized in their digestive processes as in their methods of feeding. It has been shown in Paper II of this series that Madreporaria are highly specialized carnivores, alike in their feeding mechanisms and in the nature of their digestive processes. *The stimulus of starvation cannot alter their digestive enzymes.*

Another point which Boschma emphasizes is the abundance of zooxanthellae, many of them degenerating, in the "absorptive" zone of the mesenterial filaments of starved corals, and the almost complete absence of zooxanthellae in the same region in corals which have been abundantly fed with meat. It is clear that in the first instance the zooxanthellae are in process of expulsion owing to the lowered metabolism of the coral, and that in the second case the heightened metabolism of the corals enables an increased number of zooxanthellae to live within its tissues. As a result there is no need for any to be expelled.

Even although digestion of entire zooxanthellae did not occur, the possibility remained that there might be a transference of material, especially of fatty substances which could presumably be utilized by the animal, from the zooxanthellae to the coral. According to Keeble and Gamble (1907), there is such a transference from the symbiotic *Chlamydomonas* to the tissues of *Convoluta roscoffensis*. A visit was made to Roscoff in the summer of 1930 and living *Convoluta* were examined, while subsequently sections were made of material fixed in Flemming's strong fluid. The accuracy of the original observations was confirmed. It will be demonstrated in the final paper (VII) of this series, where the conditions in *Convoluta* and in the Madreporaria will be compared, that the nature of the relationship between the animals and their contained plants in the two cases is totally different. It has been stated in this paper that all attempts to discover any transference of fat or other material from the zooxanthellae to the tissues of the corals were unsuccessful. Unlike the *Chlamydomonas* of *Convoluta*, the zooxanthellae are surrounded, as shown in Paper IV, by a thick cellulose wall which effectively prevents any such transference. The questionable statement of Arndt (1913), whose sole evidence lay in the resemblance between the staining reactions of the lipoid substances in the tissues and those in the zooxanthellae, that fat is transferred from the zooxanthellae to the tissues of the actinian, *Heliatis bellis*, was not confirmed. Of interest in this connection are the recent findings of Krogh, Lange and Smith (1931) that "the organic material synthesized by the assimilation of fresh-water algae is almost quantitatively stored in the cells of the algae, while a fraction amounting at most to 10% may possibly be lost to the surrounding water. We think it most probable that the organic substances directly lost during assimilation are wholly negligible, and that the losses observed in these experiments are mainly due to dead and decomposing organisms."

Boschma (1923), in the course of experiments on the artificial formation of buds on *Fungia fungites*, blocked the mouths of a large number of specimens with putty. Many of these lived with the putty in position for long periods, up to twelve months. This fact he has brought forward in correspondence with the senior author, as additional evidence in favour of his views that corals can obtain nourishment from their zooxanthellae. But it appears from his results that in many cases the corals formed new mouths, while, as shown in the course of this paper, the enforced starvation of the coral would lead to a shrinking

of the tissues of the disc, and so to exposure of the mesenterial filaments in the region around the putty. Moreover, in Paper II (p. 77 of this volume) it was shown that *Fungia* (No. 18) can take in living prey (in this case a Mysid) through *openings in the tissue of the disc* and, after eight hours, discharge the empty skeleton after the tissues have been completely digested by the mesenterial filaments.

One or two other results of the work recorded in this paper call for comment here. The ability of reef-building corals to exist for long periods in total darkness, already experimentally shown in Paper IV, has been further demonstrated. In some cases, notably in *Lobophyllia*, and also in *Psammocora*, as revealed by the experiments on phosphorus excretion, the corals did not live well in the light-tight box in the aquarium, but this was probably due to the experimental conditions, because this coral lived well in darkness in the light-tight box on the reef-flat (see Paper IV).

Zooxanthellae were extruded as a result of starvation in the same manner as after exposure to high temperature or to darkness, *i. e.* by way of the mesenterial filaments. They were apparently always carried in wandering cells, but examination of sections of newly-settled colonies of *Pocillopora*, which is excellent histological material, showed that they might, apparently, be contained within tissue-cells in the endoderm. The suggestion, tentatively put forward in Paper IV, that they may *always* be contained within wandering cells, cannot, therefore, be maintained. But there is no doubt that they are *always contained within cells*, either in those of the endoderm or in wandering cells.

Finally, it can be stated as a definite conclusion of this paper that the Madreporaria obtain no nutriment whatsoever from their contained zooxanthellae. The presence of the plants, as shown by the experiments on newly-settled colonies of *Pocillopora*, is not necessary for the initial budding and early growth of individual corals, although this is inhibited in the absence of animal food. Zooxanthellae are extruded from starved corals owing to the lowering of the metabolism of the animals, and a consequent diminution of the supplies of inorganic food materials for the plants. The nature of the relationship of zooxanthellae and the Madreporaria, which has already been briefly commented upon in Paper IV, will be discussed in detail in the final paper in this series.

6. SUMMARY.

1. The experiments recorded in this paper were conducted in the hope of determining definitely whether or no corals can, when starved, obtain nourishment from their zooxanthellae. This has been the subject of a great controversy in the past.

2. A series of experiments were set up in which corals were starved and fed under parallel conditions in the light and also in total darkness within an especially constructed light-light box.

3. Species of *Pocillopora* (adult colonies), *Acropora*, *Montipora* and *Porites*, all of which possess small polyps, failed to live under these conditions, and this may be explained by their need for powerful water movements.

4. The conditions in *Millepora*, which lived well, were obscure, and indicate that conditions here may be different from those in the madreporarian corals.

5. *Fungia danai* starved in the light immediately began to extrude large numbers of zooxanthellae, the majority of which, though not all, were dead. The disc tissue retreated from the mouth. This was plain after nine days' starvation and very striking after 73

days, when the greater part of the upper surface of the skeleton was exposed. *Fungia* fed in the dark had healthy, intact tissues after 165 days, but great numbers of zooxanthellae, all dead, were extruded, the tissues becoming almost colourless. Animals starved in the dark were more difficult to keep alive and behaved like those starved in the light, except that their zooxanthellae were more rapidly extruded and were all dead. Animals fed in the light remained healthy with intact tissues and the normal content of living zooxanthellae for indefinite periods up to 111 days.

6. Similar results with light-starved and dark-fed animals were obtained with *Goniastrea* sp., both living for long periods and losing their zooxanthellae, but suffering a considerable diminution of their tissues when starved. The reduction of zooxanthellae in the latter case was accompanied by an increase in the numbers of wandering cells in the endoderm.

7. Specimens of *Psammocora gonagra* lived for 228 days under all conditions and gave similar results, the coenosarc of the starved corals being greatly reduced. Examination of sections of material fixed in Flemming showed that in no case was there evidence of any digestion of the zooxanthellae, or of any transference of material from them to the tissues of the coral.

8. *Galaxea fascicularis* also gave similar results, although this coral did not feed so readily under experimental conditions.

9. Experiments with *Cyphastrea chalcidicum* were successful and gave similar results.

10. *Lobophyllia corymbosa* did not live well in the light-tight box, but showed the effect of starvation in the light almost immediately by the extrusion of immense numbers of zooxanthellae and the reduction of the edge-zone tissue, which completely disappeared after starvation for 76 days, although that of a similar polyp fed in the light was intact after 119 days.

11. Unlike adult specimens, newly settled *Pocillopora bulbosa* formed good experimental material. The fed colonies, alike in the light and the dark, grew and budded, although the latter lost their zooxanthellae. Starved colonies, whether zooxanthellae were present, as in the light, or absent, as in the dark, invariably failed to grow and bud, and those in the dark soon died. In both cases the zooxanthellae were speedily expelled and the corals became colourless and transparent.

12. *Dendrophyllia nigrescens*, which contains no zooxanthellae, was affected by the experimental conditions in exactly the same way as the other corals (except for the expulsion of zooxanthellae). The fed polyps were healthy and expanded freely after 121 days, in both light and darkness, while both starved polyps, though still alive, had greatly reduced tissues and never expanded.

13. After they had been in the experiment for 137 days the four specimens of *Psammocora gonagra* were removed, and the oxygen exchange between each of them and definite quantities of sea-water was determined for equal periods in light and in darkness. The difference between the two sets of figures showed that most zooxanthellae were contained in the light-fed coral; the specimen starved in the light contained about 64% of this number, and the two kept in the dark both about 40%. The resemblance between the figures for the two last showed that both were affected by the same adverse circumstance—lack of light—and that the zooxanthellae in the starved specimen were not further reduced in numbers owing to their digestion by the coral.

14. The excretion of phosphorus by these corals was determined after they had been

in the experiment for 218 days. This was greatest in the coral starved in the light which had few zooxanthellae but was itself healthy, though with reduced tissues. The coral fed in the light contained numerous healthy zooxanthellae which intercepted the greater portion of the phosphorus. The corals kept in the dark both gave similar figures to those obtained for the animal fed in the light. The lower excretion of phosphorus by the fed specimen indicates that confinement in the dark is not favourable to such corals.

15. Madreporaria when starved quickly show a reduction in the bulk of their tissues, and this is shown equally whether they possess zooxanthellae or not.

16. Zooxanthellae are expelled in large numbers almost immediately after starvation begins, owing to the lowered metabolism of the coral and the consequent lack of inorganic food materials for the plants. Unlike those expelled from corals kept in the dark, they are never all dead when extruded.

17. There is no evidence whatsoever of any digestion of the zooxanthellae by the corals, or any transference of material from the plants to the tissues of the animal.

18. Madreporaria obtain no nourishment from their contained zooxanthellae, nor are these necessary for the initial budding and early growth of newly-settled colonies.

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Photo M. J. Yonge. FIG. 1.

Photo G. W. Otter. FIG. 2.

Photo M. J. Yonge. FIG. 3.

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DESCRIPTION OF PLATE II.

FIG. 4.—*Fungia danai*, specimen A1 after being starved in the light for 73 days.

FIG. 5.—*Fungia danai*, specimen A4 after being starved in the light for 28 days.

FIG. 6.—*Fungia danai*, specimen B1 after being fed in the dark for 71 days.

FIG. 7.—*Fungia danai*, specimen B2 after being fed in the dark for 70 days.

FIG. 8.—*Fungia danai*, specimen D1 after being fed in the light for 79 days. The irregular shape of this coral is due to the fact that it had grown wedged between two rocks.

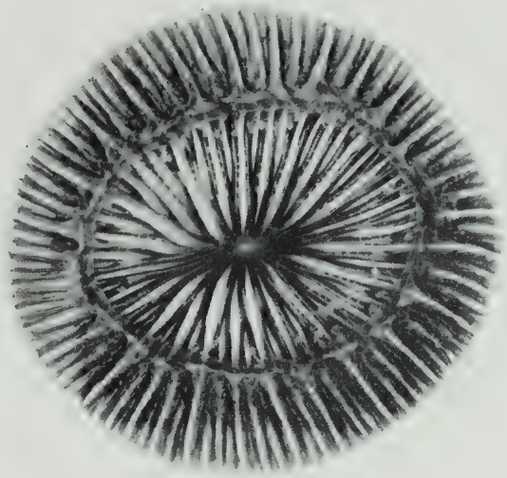


FIG. 4.

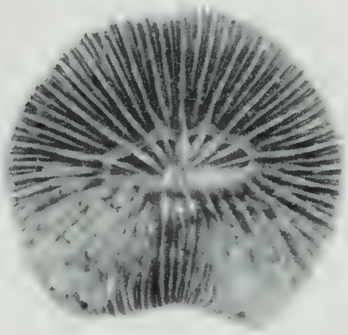


FIG. 5.

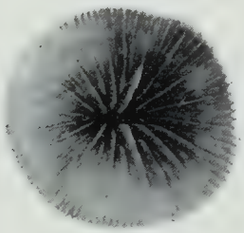


FIG. 7.

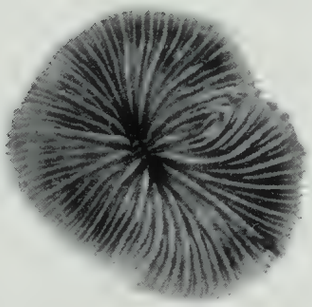


FIG. 6.

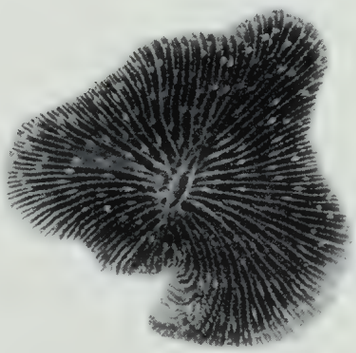


FIG. 8.

Photos G. W. Otter.

[*Adlard & Son, Ltd., Impr.*

DESCRIPTION OF PLATE III.

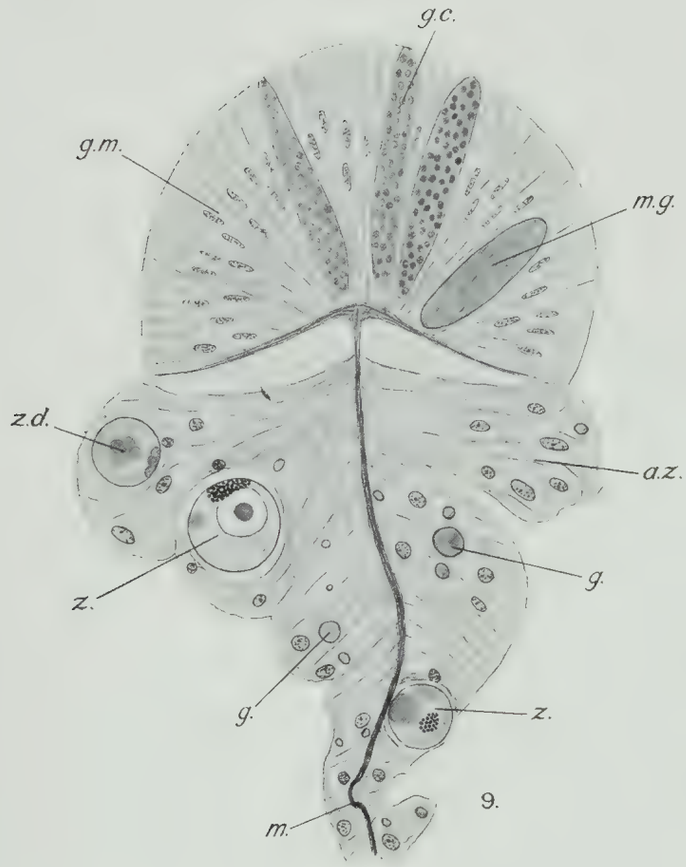
Lettering employed : *a.z.*, " absorptive " zone ; *en.*, endoderm ; *f.*, fat ; *g.*, granule staining red with safranin ; *g.c.*, gland-cell ; *g.m.*, glandular margin of mesenterial filament ; *m.*, mesogloea ; *m.g.*, mucus-gland ; *n.c.z.*, nucleus of cell enclosing zooxanthella ; *n.en.*, nucleus of endoderm cell ; *o.*, oil-droplet in zooxanthella ; *w.c.*, wandering cell ; *z.*, zooxanthella ; *z.d.*, degenerating zooxanthella.

Fig. 9.—*Psammocora gonagra*, transverse section through a mesenterial filament of specimen A1 after it had been starved in the light for 166 days. Fixed in Flemming's strong fluid, stained safranin and light green. × 1800.

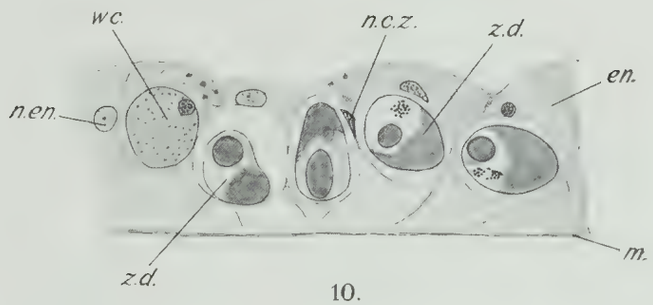
Fig. 10.—*Psammocora gonagra*, section through endoderm of coenosarc of specimen B1 after it had been fed in the dark for 92 days. Fixed in Flemming's strong fluid, stained safranin and light green. × 1800.

Fig. 11.—*Pocillopora bulbosa*, section through endoderm of disc of newly-settled specimen A1 after it had been starved in the light for 24 days. Fixed in Flemming's strong fluid, stained safranin and light green. × 1800.

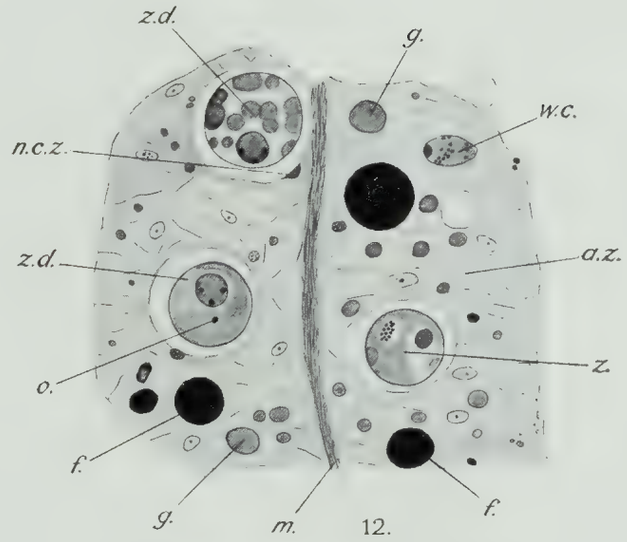
Fig. 12.—*Pocillopora bulbosa*, transverse section through " absorptive " zone of a mesenterial filament of newly-settled specimen A1 after it had been starved in the light for 24 days. Fixed in Flemming's strong fluid, stained safranin and light green. × 1800.


g.c.
g.m.
m.g.
z.d.
a.z.
z.
g.
g.
z.
m.

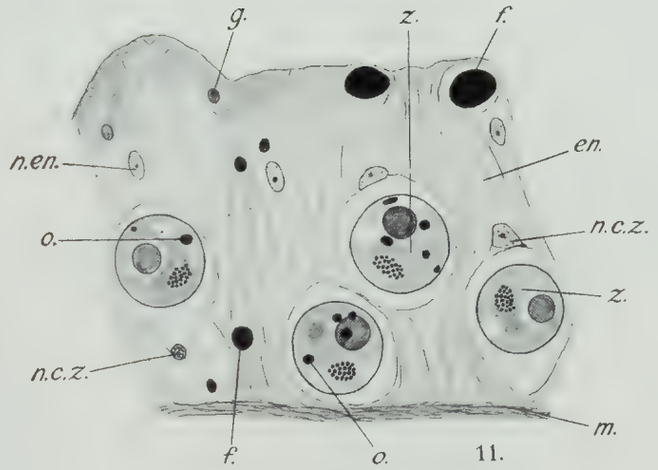
9.


w.c.
n.c.z.
z.d.
en.
n.en.
z.d.
m.

10.


z.d.
g.
w.c.
n.c.z.
z.d.
a.z.
z.
o.
f.
g.
m.
f.

12.


g.
z.
f.
n.en.
en.
o.
n.c.z.
z.
n.c.z.
m.
f.
o.

11.